Proc. Natl. Acad. Sci. USA
Vol. 77, No. 4, pp. 2333–2337, April 1980
Neurobiology

Histochemical changes in cytochrome oxidase of cortical barrels after vibrissal removal in neonatal and adult mice*

(endogenous oxidative enzymes/histochemical and cytochemical marker/functional changes/neuronal plasticity)

M. T. T. WONG-RILEY† AND C. WELT‡

†Department of Anatomy, University of California, San Francisco, California 94143; and ‡Central Wisconsin Center for the Developmentally Disabled, Madison, Wisconsin 53704

Communicated by Clinton N. Woolsey, January 31, 1980

ABSTRACT **The posteromedial barrel subfield of the somatosensory cortex of mice was examined histochemically for cytochrome oxidase activity (cytochrome *c* oxidase; ferrocytochrome *c*:oxygen oxidoreductase, EC 1.9.3.1). In normal mice, a high enzymatic activity was found within the barrel hollows, rather than in the sides and septa. Electron microscopic examination indicated that within the hollows reactive mitochondria reside in many dendrites, in some axonal terminals, and in a few neuronal perikarya. After neonatal cauterization of selected row(s) of vibrissae, the corresponding row(s) of barrels appeared as narrowed fused band(s) and their cytochrome oxidase activity was much reduced. Removal of vibrissae in the adult, by either cauterization or repeated plucking, did not cause size changes of cortical barrels. However, there was a significant decrease in the oxidative enzymatic activity within these barrels. Thus, the deprivation of sensory input through damage to, or removal of, the peripheral sensory organ induces an enzymatic response in neurons that are at least two to three synapses away from the periphery.**

The brain derives most of its energy from oxidative metabolism (1). Because there is tight coupling between energy metabolism and neuronal activity (2), it is reasonable to expect that altered activity within the central nervous system will be reflected by regional changes in levels of energy metabolism. One of us (M.W.-R.) has shown that this adjustment involves the level of activity of an important energy-deriving mitochondrial enzyme, cytochrome oxidase (cytochrome *c* oxidase; ferrocytochrome *c*:oxygen oxidoreductase, EC 1.9.3.1), in affected groups of neurons. It was found that chronic sensory deprivation of the auditory (3) or visual (4) systems resulted in a histochemically demonstrable lowering of the cytochrome oxidase activity within relay centers one to several synapses away. Moreover, this enzymatic change was reversible when the quiescent auditory nerves were functionally reactivated by electrical stimulation (5).

In the somatosensory system of rodents, there is a one-to-one relationship between the whiskers and lamina IV cortical barrels (6–8). Neonatal vibrissal damage alters the development of cortical barrels, which either fail to form or become shrunken (9–12), whereas similar procedures on postnatal day 7 or later in mice do not result in any apparent cytoarchitectural changes in the barrel field (11). The aims of the present study were: (*i*) to examine the applicability of the cytochrome oxidase technique to the study of functional changes in the somatosensory system of rodents; (*ii*) to investigate the effects of neonatal vibrissal damage on the level of enzyme activity within the corresponding cortical barrels; (*iii*) to see whether vibrissal damage in the *adult* leads to enzymatic changes within cortical barrels, even though no cytoarchitectural changes have been noted; and (*iv*) to determine if mere removal of whiskers by plucking, without cauterization, would result in any enzymatic alterations in the adult.

MATERIALS AND METHODS

Twenty-nine Swiss albino mice were used. Sixteen had vibrissal follicles electrocauterized on the right mystacial pad within a few hours after birth; four each in row C, rows B and D, rows AB and DE, and rows ABCDE were cauterized. Similar patterns of vibrissal cauterization were done in *adult mice*, two in each group. Two adult mice had their vibrissae plucked, without anesthesia, under a dissecting microscope; one had row C and the other had rows AB and DE plucked. The whiskers were examined every 2 to 3 days and replucked, if they grew back. Brains from three normal mice and the unaffected ipsilateral cerebral hemispheres served as controls. Animals were allowed to survive for 10, 12, or 16 weeks, at which time the mystacial vibrissae were rechecked under the dissecting microscope for verification of the presence or absence of hair follicles and for any signs of regrowth. The animals were then anesthetized with ether and perfused with phosphate-buffered saline followed by cold 2.5% (wt/vol) paraformaldehyde and 1.5% (wt/vol) glutaraldehyde in 0.1 M sodium phosphate buffer and 4% sucrose. The facial pads were examined histologically with hematoxylin and eosin to confirm the sites of cauterization. The brains were removed immediately and placed in the fixative for 1 hr, then in sucrose/buffer for several hours or overnight. Serial frozen or vibratome sections were cut at 60 μm thickness, tangential to the pia overlying the barrel field. Sections from the experimental and control hemispheres of each animal were always incubated simultaneously in the same medium. The protocol for the cytochrome oxidase histochemical reaction was the same as stated previously (4). Some sections were counterstained with 1% cresyl violet to reveal cell bodies. Histochemical changes were identified on all consecutive sections to differentiate between true histochemical changes and a partial image of a barrel grazed in sectioning. For electron microscopic study, vibratome-cut sections were incubated and initially examined under both the compound and dissecting microscopes. The precise *single* barrels or fused bands were identified and carefully dissected out, fixed with osmium tetroxide, and processed as described (4).

RESULTS

The posteromedial barrel subfield (PMBSF) (6), representing the large contralateral mystacial vibrissae, is easily identified by the technique described above (Fig. 1). Nissl counterstaining shows that heightened cytochrome oxidase activity occurs

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: PMBSF, posteromedial barrel subfield.

* Portions of this work were presented at the Ninth Annual Meeting of the Society for Neuroscience, November 1979.

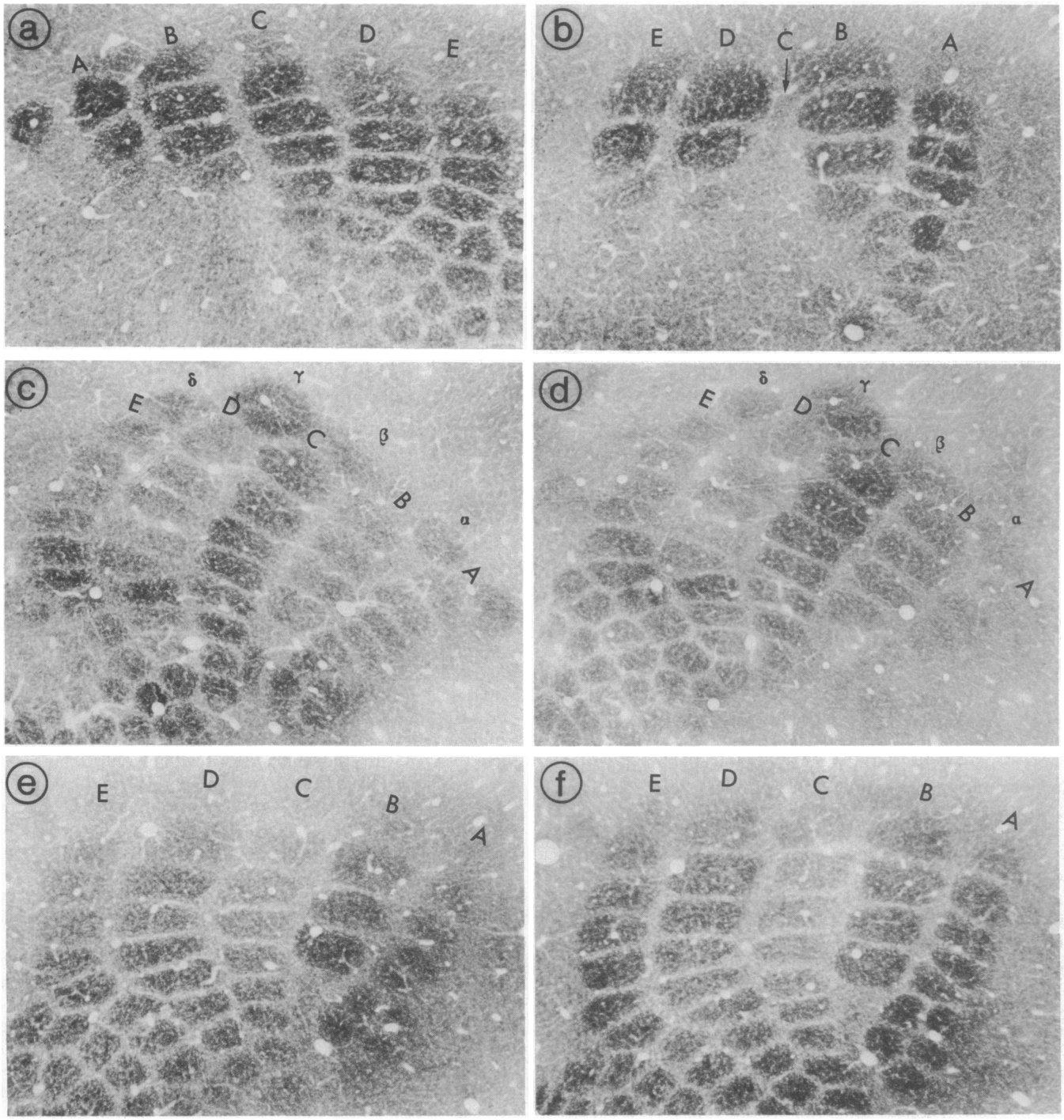

FIG. 1. (*a*) Normal PMBSF in lamina IV of mouse SmI cortex stained for cytochrome oxidase activity. Note the darkly reactive barrel hollows compared with considerably less reactive barrel sides and septa. (×35.) In this and *b–f*, letters A–E designate barrel rows. The single barrel adjacent to row A corresponds to a sinus hair above the eye. Medial is up and anterior is toward row E. Because *a* is taken from the control (right) hemisphere, it appears as a mirror image to *b–f*, which are taken from the experimental (left) hemispheres.

(*b*) PMBSF of SmI cortex contralateral to mystacial pad with row C cauterized at birth. The animal survived for 87 days. Note that the dewhiskered cortical row C appears as a slender band of low cytochrome oxidase activity. The barrels adjacent to row C, particularly those in rows B and D, are larger than normal and have high enzyme activity. (×35.)

(*c*) PMBSF of SmI cortex contralateral to mystacial pad with rows A-1–5, B-1–4, D-1–5, E-1–4, and straddlers α, β, and δ cauterized in the *adult*. The animal survived for 62 days postoperatively. Note the decreased cytochrome oxidase activity in the dewhiskered barrels. Row C, γ straddler, and barrels in the other rows that exhibit high enzyme activity represent intact vibrissae that were not cauterized. (×35.)

(*d*) Section immediately adjacent to (above) the one in *c*. It demonstrates that row C and γ straddler remain darkly reactive, while the dewhiskered barrels remain less reactive than normal. Changes in dewhiskered barrels are seen consistently in consecutive sections throughout lamina IV, and can be easily differentiated from occasional light staining due to grazing sections. (×35.)

(*e* and *f*) Two consecutive sections showing PMBSF of SmI cortex contralateral to mystacial pad with row C repeatedly *plucked* in the *adult* for 80 days prior to sacrifice. Note the decreased cytochrome oxidase activity in the dewhiskered row C, while the enzyme activity in the other rows remains high. (×35.)

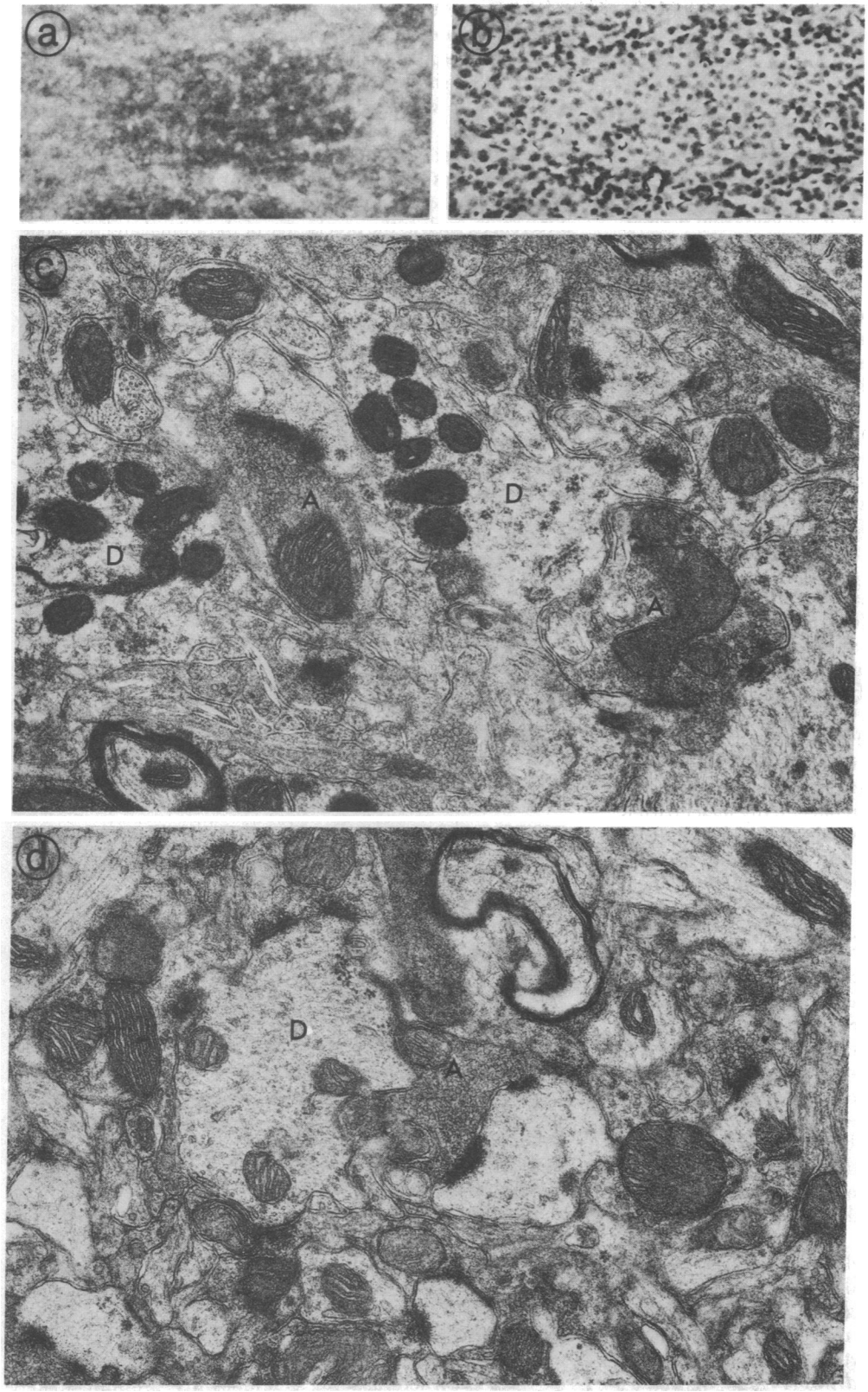

FIG. 2. (*a*) A single barrel (C-5) from normal PMBSF is shown after the tissue has been stained for cytochrome oxidase activity. Note that the barrel hollow is highly reactive, whereas the sides and septa are much less reactive. (×105.)

(*b*) The barrel in *a* is shown after Nissl counterstaining. Note the corresponding positions of the blood vessel in *a* and *b*. The ring of neurons forming the sides of the barrel clearly lies outside of the highly reactive barrel hollow as seen in *a*. This photograph was taken with a Kodak Wratten no. 25 filter to partially suppress the brown staining of the reaction product in the hollow so that the Nissl-stained cell bodies within the hollow and sides can be more easily visualized. (×105.)

(*c* and *d*) Both electron micrographs are taken from synaptic regions within the barrel hollows of normal mouse PMBSF. *d* is a control from unincubated tissue. Compare the mitochondria within dendritic (D) and axonal (A) profiles in *d* with those in *c*, which is taken from incubated material. In *c*, note that the dendritic processes labeled D contain very reactive mitochondria, whereas the mitochondria within the two axonal terminals marked A are much less reactive. Reactive mitochondria are seen in some axonal terminals not depicted in this figure. (×27,340.)

mainly within the barrel hollows, whereas barrel sides, septa, septal junctions, and neuronal cell bodies forming the sides of the barrels generally are not highly reactive (Figs. 1 and 2 *a* and *b*). Thus, there is a sharp boundary between the markedly reactive barrel hollow and the weakly reactive barrel sides. In the electron microscope, as reported before (4), reaction products are localized mainly along the outer surface of the inner mitochondrial membranes, including the intracristate space of reactive mitochondria. The barrel hollows are seen to contain reactive mitochondria occurring in many *dendritic* processes (compare Fig. 2 *c* and *d*), in some axonal processes, and in a number of neuronal perikarya. Most neuronal cell bodies in the

barrel sides have mitochondria with relatively low amounts of reaction product, but a few scattered neurons do contain very reactive mitochondria.

In the experimental hemispheres, distinct changes can be seen in the pattern of cytochrome oxidase staining of the PMBSF. In mice with vibrissae cauterized shortly after birth, the corresponding row or rows of cortical barrels are severely reduced in size. In the abnormal regions associated with the damaged whiskers, a fused slender band or bands of cytochrome oxidase activity appear(s) much reduced from normal (Fig. 1*b*). The pattern is similar to the fused bands of succinate dehydrogenase staining described in the PMBSF of rats with neonatal vibrissal cauterization (13). In addition, the cytochrome oxidase activities within the abnormal bands are consistently lower than those in barrels of the normal controls (compare Figs. 1 *a* and *b*). Barrels adjacent to these altered regions usually increase in size and sustain a high level of enzymatic activity.

When vibrissae are cauterized in adult mice, the effects are different. In this case, the corresponding cortical barrels do not show any changes in size, location, or arrangement. However, *the cytochrome oxidase activity in these barrel hollows is markedly reduced* (Fig. 1 *c* and *d*). Repeated plucking of vibrissae without cauterization in the adult also results in a much *decreased* enzyme activity in the corresponding, cytoarchitecturally intact, barrels (Fig. 1 *e* and *f*).

These results demonstrate that: (*i*) cytochrome oxidase histochemistry can be reliably used as an anatomical marker that correlates with the known cytoarchitectonic pattern in the somatosensory cortex of rodents; (*ii*) after neonatal damage of the vibrissal follicles, the pattern of cytochrome oxidase activity parallels the altered organization of the PMBSF, and, in addition, there is a significant decrease in the cytochrome oxidase activity within the affected zones; (*iii*) whisker damage in the adult causes alterations within cytoarchitecturally intact barrels that are reflected by the decreased cytochrome oxidase activity; and (*iv*) repeated vibrissal removal in adult mice, without electrocauterization, is sufficient to cause enzymatic changes at the cortical level.

DISCUSSION

The findings presented here should be considered in several contexts. First, cytochrome oxidase activity in *normal* mice has been demonstrated directly to be much higher in the barrel hollows than in the sides and septa. The correspondence between the overall pattern of cytochrome oxidase activity and the known cytoarchitecture of barrels implies that the major site of synaptic interactions between thalamic afferents and cortical neurons (14, 15), that is, the barrel hollow, has a greater oxidative metabolic activity than the surrounding barrel regions. The pattern is also similar to that shown by the histochemical reaction of succinate dehydrogenase, which is another mitochondrial enzyme intimately associated with electron transport and oxidative phosphorylation. However, whereas the light microscopic succinate dehydrogenase studies on barrels (13, 16) have emphasized the localization of the stain to thalamocortical terminals, our electron microscopic data indicate a significant contribution to the cytochrome oxidase activity from mitochondria in cortical *dendrites* (Fig. 2*c*). As yet, we do not know if the reactive axonal terminals in our study belong to thalamic or other sources. The presence of both reactive and nonreactive dendrites and neuronal perikarya within the barrels suggests the possibility of at least two classes of neurons, each with a different level of oxidative metabolism. This is of interest, because it has been reported that there are two principal classes of barrel neurons with different morphological and physiological characteristics (17, 18).

Second, the altered pattern of barrels revealed by the cytochrome oxidase method in animals with neonatal whisker damage corresponds to other published reports. The arrangement that we show (Fig. 1*b*) resembles that published by Van der Loos and Woolsey (9) rather than that shown by Woolsey and Wann (11). In the former report, the barrelless regions still showed slender "sheetlike assemblies of cells," whereas in the latter there was "virtually no zone for row C," when vibrissae were damaged on postnatal day 1. Some possible bases for these differences, such as the precise age of the animal and the mode and extent of peripheral lesions, have been discussed elsewhere (11). Our findings of a *fused band* of oxidative enzymatic activity within the dewhiskered zone support the description of Killackey *et al.* (13), who used the succinate dehydrogenase method. However, the basic difference is that we found a *reduction* in the enzymatic activity, whereas no enzymatic changes were reported in their study. The reason for this difference is unclear, but unpublished data from mice prepared in a manner similar to ours do indicate a reduction of succinate dehydrogenase activity in the altered cytoarchitectonic zone (D. Durham, personal communication).

Third, the principal finding in this study is that removal of whiskers in *adult* animals, either by electrocauterization or *repeated plucking*, results in decreased cytochrome oxidase activity in the appropriate barrels (Fig. 1 *c–f*). The observations in the cauterized cases are of interest, because no cytoarchitectural changes have been detected in other studies, when vibrissae were damaged after postnatal day 7, at which time the cortex was thought to have become refractory to the effect of peripheral damage (10–12). Our electron microscopic results suggest that the effect of chronic sensory deprivation may be manifested in cortical neurons. Perhaps more important is the finding of *decreased cytochrome oxidase activity* in the barrels after chronic removal of vibrissal hairs alone, which represents a response of a neural system of *reduced functional activity* at least *three synapses away from the periphery*. In this regard, it is important to clearly point to the difference between a reduction in enzymatic activity, which we report, and a reduction of glucose utilization, which has been reported in 2-deoxyglucose studies of this system after acute whisker removal (19). The two studies show a biochemical response to altered neuronal functional states. Whereas the 2-deoxyglucose method indicates a prompt change in glucose utilization, the present cytochrome oxidase approach reveals an enzymatic adjustment of the neuronal population to a chronically lowered functional demand that has presumably reached a steady state. The latter problem is of general interest, because it provides a link between the neurobiologist's concern with excitable cells and the cell biologist's concern with how the metabolic machinery necessary for this excitability is regulated.

Finally, this study establishes a base line for further studies of the mechanisms of change in cytochrome oxidase levels, particularly with respect to sensory deprivation in adult animals. One obvious study is to examine the time course of change in cytochrome oxidase activity. This would provide data about the rate at which the neuronal regulation of this enzyme proceeds. A second approach is to define the enzymatic changes at the ultrastructural level. Questions that are immediately relevant are: (*i*) What percentages of reactive mitochondria are in dendrites, in axonal trunks and terminals, and in perikarya of neurons within the barrel hollows? Morphometric analysis at the electron microscopic level can be applied. (*ii*) What specifically accounts for the decrease in the cytochrome oxidase activity? Does it represent a decrease in the total number of mitochondria, a decrease in the relative activity of enzymes in each mitochondrion, or a combination of the above in various

neuronal profiles? The answers to these questions should provide useful information with respect to the cortical barrels and to the cytochrome oxidase method, as well as to the mechanism responsible for the regulation of enzymatic activity in neurons in response to altered functional demands.

It is our great pleasure to thank Dr. Thomas A. Woolsey for his critical review of this manuscript and for his valuable suggestions. We also thank Miss D. Crumrine, Mrs. S. Thompson, Mr. D. Snyder, and Mr. D. Akers for their expert technical help. This study was partially supported by National Institutes of Health Grant NS-12995 and by the State of Wisconsin.

1. Krnjević, K. (1975) in *Brain Work*, Alfred Benzon Symposium 8, eds. Ingvar, D. H. & Lassen, N. A. (Academic, New York), pp. 65–78.
2. Lowry, O. H. (1975) in *Brain Work*, Alfred Benzon Symposium 8, eds. Ingvar, D. H. & Lassen, N. A. (Academic, New York), pp. 48–64.
3. Wong-Riley, M. T. T., Merzenich, M. M. & Leake, P. A. (1978) *Brain Res.* **141**, 185–192.
4. Wong-Riley, M. (1979) *Brain Res.* **171**, 11–28.
5. Wong-Riley, M. T. T., Walsh, S. M., Leake-Jones, P. A. & Merzenich, M. M. (1979) *Ann. Otol. Rhinol. Laryngol. Suppl.*, in press.
6. Woolsey, T. A. & Van der Loos, H. (1970) *Brain Res.* **17**, 205–242.
7. Welker, C. (1971) *Brain Res.* **26**, 259–275.
8. Simons, D. J. & Woolsey, T. A. (1979) *Brain Res.* **165**, 327–332.
9. Van der Loos, H. & Woolsey, T. A. (1973) *Science* **179**, 395–398.
10. Weller, W. L. & Johnson, J. I. (1975) *Brain Res.* **83**, 504–508.
11. Woolsey, T. A. & Wann, J. R. (1976) *J. Comp. Neurol.* **170**, 53–66.
12. Jeanmonod, D., Rice, F. L. & Van der Loos, H. (1977) *Neurosci. Lett.* **6**, 151–156.
13. Killackey, H. P., Belford, G., Ryugo, R. & Ryugo, D. K. (1976) *Brain Res.* **104**, 309–315.
14. White, E. L. (1976) *Brain Res.* **105**, 229–251.
15. White, E. L. (1978) *J. Comp. Neurol.* **181**, 627–661.
16. Killackey, H. P. & Belford, G. R. (1979) *J. Comp. Neurol.* **183**, 285–304.
17. Woolsey, T. A., Dierker, M. L. & Wann, D. F. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2165–2169.
18. Simons, D. J. (1978) *J. Neurophysiol.* **41**, 798–820.
19. Durham, D. & Woolsey, T. A. (1978) *J. Comp. Neurol.* **178**, 629–644.